# Correlation between alterations in blood flow of malignant lymphomas after induction chemotherapies and clinical outcomes: A pilot study utilizing contrast-enhanced ultrasonography for early interim evaluation of lymphoma treatment

日本大学医学部内科学系消化器肝臓内科学分野

熊川　まり子

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指導教員　　森山　光彦

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# Correlation between alterations in blood flow of malignant lymphomas after induction chemotherapies and clinical outcomes: a pilot study utilising contrast-enhanced ultrasonography for early interim evaluation of lymphoma treatment

M. Kumagawa [a], N. Matsumoto [a,*], K. Miura [b,**], M. Ogawa [a], H. Takahashi [b], Y. Hatta [b], R. Kondo [c], N. Koizumi [c], M. Takei [b], M. Moriyama [a]

[a] *Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, 30-1, Oyaguchi Kami-cho, Itabashi-ku, Tokyo, Japan*

[b] *Division of Hematology and Rheumatology, Department of Medicine, Nihon University School of Medicine, 30-1, Oyaguchi Kami-cho, Itabashi-ku, Tokyo, Japan*

[c] *Department of Mechanical Engineering and Intelligent Systems, Graduate School of Informatics and Engineering, The University of Electro-Communications, 1-5-1, Chofugaoka, Chofu-shi, Tokyo, Japan*

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AIM: To clarify the utility of contrast-enhanced ultrasonography (CEUS) for interim evaluation of response to chemotherapy in lymphoma treatment.

MATERIALS AND METHODS: CEUS was performed both before (day 0) and after the treatment (7 and/or 14 days), and a time–intensity curve was obtained. The patients were divided into two groups (complete remission [CR] group and non-CR group) according to the results of conventional response evaluation, and peak enhancement (PE), time to peak enhancement, perfusion index (PI), the total area under the curve during wash-in (AUC-in), and the total AUC were compared between the groups.

RESULTS: Among 27 patients with various types of lymphoma, the median change ratio of PE and PI at day 7 evaluation were significantly different between the CR group and the non-CR group (0.81 versus 1.39, $p$=0.017 for PE and 0.92 versus 2.09, $p$=0.010 for PI). The change ratio of PE $<$ 1.09 (specificity: 86%; sensitivity, 88%) and PI $<$ 1.65 (specificity: 86%; sensitivity: 94%) distinguished CR from non-CR. Patients who achieved a PE change ratio $<$1.09 or a PI change ratio $<$1.65 had significantly better estimated progression-free survival ($p$$<$0.001).

* Guarantor and correspondent: N. Matsumoto, Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, 30-1, Oyaguchi Kami-cho, Itabashi-ku, Tokyo, Japan. Tel.: +81 3 3972 8111; fax: +81 3 3972 0015.

** Guarantor and correspondent: K. Miura, Division of Hematology and Rheumatology, Department of Medicine, Nihon University School of Medicine, 30-1, Oyaguchi Kami-cho, Itabashi-ku, Tokyo, Japan. Tel.: +81 3 3972 8111; fax: +81 3 3972 0015.

*E-mail addresses:* matsumoto.naoki@nihon-u.ac.jp (N. Matsumoto), miura.katsuhiro@nihon-u.ac.jp (K. Miura).

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CONCLUSION: The present study demonstrated that changes in tumour perfusion parameters evaluated with CEUS at 1 week after the treatment initiation were significantly different between lymphoma patients in CR group and non-CR group. Alterations in perfusion parameters evaluated via CEUS could impact the prognosis of lymphoma patients.



## Introduction

Lymphomas are the seventh most common cancer worldwide, but they are also among the most chemotherapy-responsive malignancies, which has contributed to the decrease in lymphoma-associated mortality in the last decade.[1] Lymphomas arise from different stages of differentiation of the immune system, so their histological classification is diverse. Many clinical symptoms and imaging findings are common to various lymphomas, and one of the main symptoms is lymphadenopathy and tumour formation that progresses without spontaneous pain. In lymphomas, the time to achieve complete remission (CR) is a prognostic factor, and the longer the period, the higher the rate of recurrence; however, the degree of tumour reduction is influenced by the size and location of the tumour before treatment, histological type, and treatment method, and thus the time to achieving CR may be an insufficient prognostic factor. Moreover, it is difficult to evaluate fibrosis and the viable site in the remaining tumour shadow by measuring the size at computed tomography (CT) alone. Because 2-[$^{18}$F]-fluoro-2-deoxy-D-glucose positron-emission tomography (FDG-PET) has the ability to distinguish between viable tumour and non-metabolic mass, and evaluate systemically without being affected by tumour localisation, combined PET and CT (PET/CT) is usually used for pre-treatment staging and evaluation of response to treatment in lymphoma patients.[2,3] The goal of treatment for aggressive lymphomas, such as Hodgkin's lymphoma (HL) or aggressive non-Hodgkin's lymphoma (NHL), is increasing the cure rate and reducing mortality. Although evaluating response to treatment via PET/CT and achieving complete remission are essential, the change in tumour size obtained radiologically is late as the timing for determining the therapeutic effect. Recently, interim PET has been introduced in the management of lymphomas as a method for evaluating treatment response. Several studies have proven that PET findings during the treatment predicted clinical outcomes in HL and diffuse large B-cell lymphoma (DLBCL)[4,5]; however, there remains concerned for radiation exposure and high cost in repeated evaluation using PET/CT.

Contrast-enhanced ultrasonography (CEUS), which has been mainly used for the diagnosis of hepatic nodules, has the advantage of high spatial and time resolution, low frequency of adverse effects, absence of radiation exposure, and wide availability.[6–9] CEUS can be performed even in patients with renal dysfunction or allergy to iodine-based contrast agents.[10] It can be used to measure tumour size and contrast agent uptake accurately, and so CEUS has been also used to evaluate treatment response in various malignancies[11–17]; however, few studies have investigated the performance of CEUS for assessing early treatment response in lymphomas. Thus, the aim of the present study was to clarify the feasibility of CEUS for early, interim evaluation of response to chemotherapy in various lymphoma subtypes independent of the chemotherapy regimen.

## Materials and methods

### *Patients*

This was a prospective observational study conducted between February 2013 and June 2017 for the purpose of evaluating the value of CEUS evaluation of the treatment response in patients with various lymphomas. The inclusion criteria were age >20 years, the pathological diagnosis of lymphoma, at least one detectable lesion via ultrasonography, hospitalisation during the study period, and written informed consent. Meanwhile, the exclusion criteria were allergy to the ultrasonography contrast agent or egg and assumed unsuitable for enrolment at the physician's discretion. The International Prognostic Index was used for clinical prognostic scoring,[18] and pathological diagnosis was according to the World Health Organization classification.[19]

### *Ultrasonography scanning technique*

B-mode ultrasonography and CEUS were scheduled as follows: before the treatment (day 0), 7 days after the treatment (day 7), and 14 days after the treatment (day 14). In principle, undergoing the full three sets of CEUS was encouraged, but choosing only either day 7 or 14 as a follow-up was permitted according to the patient preference. Ultrasonography was performed using Aplio MX (Canon Medical Systems Corporation, Tochigi, Japan), and a 3.5-MHz convex transducer (PVT-375BT). A single target lesion, which could be depicted on one screen and showed the least mobility from breathing, was selected for the response evaluation. For each examination, a morphological study was performed in B-mode with a measurement of the two largest diameters of the lesion. CEUS was used for functional evaluation. Briefly, a 0.5-ml bolus of perfluorobutane microbubbles (Daiichi-Sankyo, Tokyo, Japan) was injected into the antecubital vein via a 22-G peripheral intravenous cannula, followed by a 10-ml saline flush. The recording was started at the time of contrast agent injection. Raw data were acquired in 1 minute, and the mechanical index was set at 0.2. CEUS was performed at a rate of 15 frames/second and with a dynamic range of 45 dB. Receiver gain and image depth were optimised for each patient at baseline examination. Transit focus was set at the

bottom of the target lesion. These settings were kept identical in each patient in the follow-up examinations. All CEUS evaluations were performed by three investigators who had 8–16 years of experience.

*Perfusion parameter analysis*

Raw data were retrieved from the workstation and then quantified on the computer using the original time–intensity curve (TIC) analysis software developed by R.K. Because the targets were tumours, the target areas were assumed to be elliptical. Specifically, the area of the ellipse centre was selected. Next, the lengths of the longitudinal and transverse axes of the ellipse on the ultrasound image were set such that the elliptical region contained the tumour. Finally, TIC analysis was performed by calculating the average luminance in the selected elliptical region for each frame. The regions of interest (ROIs) surrounding the lesion, including the lymph nodes, were defined. Changes in perfusion imaging of ROIs on CEUS were expressed as TICs, which were calculated as the sum of TICs of all pixels using linear raw data obtained via the original software and the proportion to the real perfusion of the target lesion. The arithmetic operation was conducted on 900 images acquired during each examination at 15 images/s for 1 min (Electronic Supplementary Material Fig. S1). Five quantitative perfusion parameters were determined based on TICs, namely, peak enhancement (PE), time to peak enhancement (TTP), perfusion index (PI), the total area under the TIC during wash-in (AUC-in), and the total AUC. PE, AUC-in, and total AUC corresponded to blood volume, whereas TTP and PI corresponded to blood flow (Fig 1). ROI selection and TIC analysis were performed by two operators. The perfusion parameters calculated by the two operators were very strongly correlated ($\rho = 0.930$, $p=2.24 \times 10^{-6}$ for PE and $\rho = 0.897$, $p=2.70 \times 10^{-6}$ for PI).

*Measurement of clinical outcomes*

The clinical prognostic scoring systems used for each patient to evaluate progression and count risk factors were based on the International Prognostic Score (IPS) for HL, the International Prognostic Index (IPI) for non-Hodgkin's lymphoma, and Follicular Lymphoma Prognostic Index (FLPI) for follicular lymphoma (FL). PET/CT imaging was performed before the start of chemotherapy and after the completion of the planned chemotherapy. Treatment response was categorised into CR, partial remission (PR), stable disease (SD), or progressive disease (PD) according to the standard criteria.[2] Progression-free survival (PFS) was defined as the time from treatment initiation to disease progression. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Clinical data, including serological and follow-up data, were obtained from the hospital records in all cases. The study was approved by the institutional review board (RK-130208-1). Informed consent was obtained from all individual participants included in the study.

*Statistical analysis*

The patients were divided into the CR group or the non-CR group according to the results of PET/CT for response evaluation. Variations for each CEUS perfusion parameter was calculated as the ratio of the value on each day of treatment (days 7 and 14) to the baseline value (day 0). Variations between the CR and non-CR groups were compared using the Wilcoxson rank-sum test. The receiver operating characteristic (ROC) curve analysis was used to determine the diagnostic power of the parameters. PFS between the two groups was compared using the log-rank test. A $p$-value of $<0.05$ was considered statistically significant. All statistical analyses were performed using the JMP software version 8.0.1 (SAS Institute, Cary, NC, USA).

## Results

*Patient characteristics*

A total of 41 patients were enrolled in the study. Of these, nine patients did not undergo CEUS after the initiation of the treatment because of withdrawal of consent

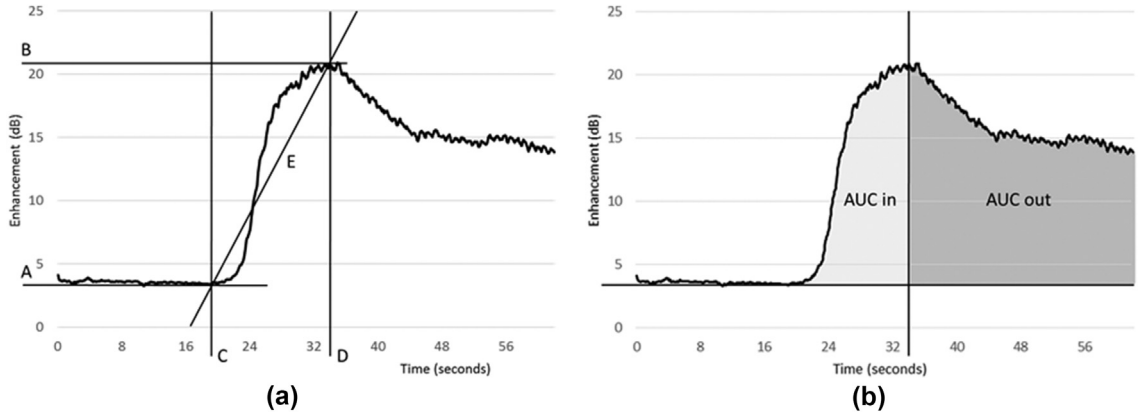


**Figure 1** (a) A representative TIC and quantitative perfusion parameters. PE equals B–A (dB); TTP equals D–C (seconds); and PI equals the slope E (PE/TTP; dB/s). (b) The AUC-in and AUC out. The total AUC equals AUC in + AUC out.

($n$=1), incomplete execution of CEUS schedule ($n$=2), severe illness ($n$=3), no ROIs detected ($n$=2), and no treatment initiated ($n$=1). Furthermore, TICs could not be depicted in five patients because the target lesion moved during their breathing. All 14 patients were excluded, and thus 27 patients (18 men and nine women) with a median age of 66 years were evaluable for the final analysis. The histological types of lymphoma consisted of DLBCL ($n$=17), FL ($n$=3), anaplastic large cell lymphoma ($n$=2), HL ($n$=1), nodal marginal zone lymphoma (NMZL; $n$=1), mantle cell lymphoma (MCL; $n$=1), angio-immunoblastic T-cell lymphoma ($n$=1), and enteropathy-associated T-cell lymphoma ($n$=1). Patients with newly diagnosed DLBCL ($n$=14), T-cell lymphomas ($n$=4), FL ($n$=2), and NMZL ($n$=1) were treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP)-based chemotherapies with or without rituximab. Patients with relapsed/refractory DLBCL ($n$=2) and FL ($n$=1) were administered rituximab, ifosfamide, etoposide, cytarabine, and dexamethasone treatment. One patient with relapsed DLBCL was administered gemcitabine, carboplatin, dexamethasone, and rituximab treatment. One patient with MCL received rituximab and bendamustine. One patient with HL received doxorubicin, bleomycin, vinblastine, and dacarbazine treatment. At the end of the chemotherapies, all 27 patients were evaluated using the standardised response criteria for HL and non-HL.[3] Accordingly, 18 and nine patients were classified into the CR and non-CR groups, respectively (Fig 2). The distributions of the patient characteristics in the CR and the non-CR groups were not significantly different (Table 1).

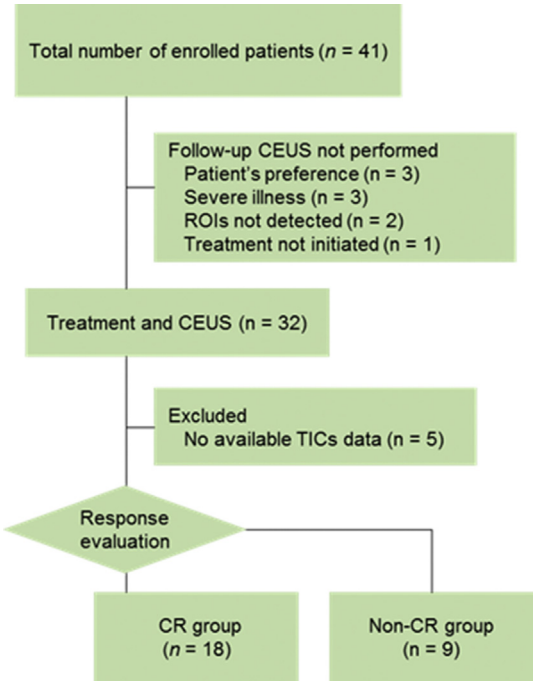


**Figure 2** CONSORT diagram of the study. Of the 41 patients, 32 underwent CEUS during treatment, and 27 were finally evaluated. They were stratified into either the CR group or the non-CR group according to the treatment response.

### *Perfusion parameters*

In total, 11 patients underwent the complete set of CEUS on days 0, 7, 14, while 13 patients were examined on days 0 and 7, and the remaining three patients were examined on days 0 and 14. The selected ROIs were intra-abdominal lymph nodes ($n$=13), cervical lymph nodes ($n$=9), axillary lymph nodes ($n$=1), inguinal lymph node ($n$=1), ileum ($n$=1), liver ($n$=1), and mammary gland ($n$=1). All patients in the study experienced a decrease in lesion size at a median ratio of 48% (range, 0–77%) as determined via B-mode ultrasonography on days 7 or 14. The median ratio of size reduction was higher in the CR group than that in the non-CR group, but the difference was not significant (49% versus 33%, $p$=0.491). The median scores of PE, PI, AUC-in, and AUC tended to decrease in the CR group after the treatment, whereas these scores increased in the non-CR group (Table 2). Meanwhile, the median score of TTP in the non-CR group became shorter after the treatment. Thus, the median PI in the CR group decreased, whereas that in the non-CR group increased after the treatment. Particularly, the median change ratio of PE and PI at day 7 evaluation was significantly different between the CR group and the non-CR group (0.81 versus 1.39, $p$=0.017 for PE and 0.92 versus 2.09, $p$=0.010 for PI; Fig 3).

### *Clinical outcomes*

To predict CR, cut-off values of changes in the ratio of PE and PI between days 0 and 7 that showed high specificity and sensitivity in correlation with CR were calculated. Accordingly, changes in the ratio of PE $<$ 1.09 (specificity: 86%; sensitivity, 88%; area under ROC, 0.82) and changes in the ratio of PI $<$ 1.65 (specificity: 86%; sensitivity, 94%; area under ROC, 0.84) distinguished CR from non-CR accurately. When these cut-off values were applied to the 24 patients who underwent CEUS on day 7, the estimated PFS for patients who obtained changes in the ratio of PE $<$ 1.09 for or of PI $<$ 1.65 was significantly better than that for the other patients (Fig 4). Patient characteristics and images of typical CR group case with favourable changes in CEUS on day 7

**Table 1**
General characteristics of the study population.

| Characteristic | CR group ($n$=18) | Non-CR group ($n$=9) | $p$-Value |
|---|---|---|---|
| Sex, men/women | 11/7 | 7/2 | 0.667 |
| Median age, years (range) | 67.5 (47–80) | 65 (49–79) | 0.439 |
| ECOG PS $\geq$ 2, $n$ (%) | 4 (22%) | 3 (33%) | 0.653 |
| Stage III/IV, $n$ (%) | 12 (67%) | 7 (78%) | 0.676 |
| LDH $>$ normal, $n$ (%) | 11 (61%) | 8 (89%) | 0.201 |
| Extranodal sites $\geq$2, $n$ (%) | 5 (28%) | 3 (33%) | 1 |
| Risk factors $\geq$3, $n$ (%) | 10 (56%) | 6 (67%) | 0.692 |
| Histology, B-NHL/others | 16/2 | 6/3 | 0.295 |

CR, complete remission; ECOG PS, Eastern Cooperative Oncology Group performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index; B-NHL, B-cell non-Hodgkin lymphomas.

**Table 2**
Distribution of perfusion parameters according to the timing of CEUS evaluation.

| | Day 0 | Day 7 | Day 14 |
|---|---|---|---|
| | CR group ($n$=18) | CR group ($n$=17) | CR group ($n$=8) |
| | Non-CR group ($n$=9) | Non-CR group ($n$=7) | Non-CR group ($n$=6) |
| Peak enhancement (dB) | | | |
| CR group, median (range) | 15 (0.71–27.32) | 12.26 (0.46–22.64) | 9.63 (3.54–17.2) |
| Non-CR group, median (range) | 16.22 (1.95–21.96) | 20.9 (7.83–26.35) | 19.3 (12.90–24.4) |
| Time to peak enhancement (s) | | | |
| CR group, median (range) | 9.33 (6.27–13) | 9.07 (7.07–16.3) | 9.9 (6.20–18.3) |
| Non-CR group, median (range) | 10.3 (6.67–13.1) | 7.53 (6.33–9.93) | 6.77 (6.27–15.4) |
| Perfusion index (dB/s) | | | |
| CR group, median (range) | 1.39 (0.09–3.92) | 1.28 (0.05–2.31) | 1.18 (0.19–2.07) |
| Non-CR group, median (range) | 1.57 (0.29–3.17) | 2.26 (1.14–4.16) | 2.28 (1.35–3.89) |
| AUC-in | | | |
| CR group, median (range) | 1,228 (53–2,610) | 1,112 (175–1,959) | 831 (370–1,328) |
| Non-CR group, median (range) | 1,412 (104–2,526) | 1,527 (471–1,678) | 1,208 (823–2,310) |
| Total AUC | | | |
| CR group, median (range) | 7,395 (803–15,401) | 5,509 (687–11,464) | 4,072 (1,445–8,064) |
| Non-CR group, median (range) | 8,753 (568–10,163) | 9,450 (2,277–13,222) | 10,409 (5,044–12,220) |

CEUS, contrast-enhanced ultrasonography; CR, complete response; AUC, total area under the time–intensity curve.

and good response to treatment, and representative cases of the non-CR group with undesired changes on day 7 and poor response to treatment were presented in Figs. 5 and 6. In addition, each video is available as Electronic Supplementary Material (Video S1; pre of CR case, Video S2; day 7 of CR case, Video S3; pre of non-CR case, Video S4; day 7 of non-CR case).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.crad.2021.02.007

## Discussion

The present study demonstrated that changes in tumour perfusion parameters evaluated with CEUS at 1 or 2 weeks after the treatment initiation were significantly different between lymphoma patients who achieved CR and those who did not. By contrast, the changes in the ratio of the target lesion size were not significantly different between these two groups. These findings suggest that decreases or increases in blood flow volume and velocity in the lesion 1 week after the start of chemotherapy could be predictors of treatment response and the other clinical outcomes such as PFS among patients with lymphomas.

Recently, the utility of quantitative CEUS for determining early response to antiangiogenic therapies has been reported in various solid tumours such as hepatocellular carcinoma,[11,12] renal cancer,[13,14] liver metastasis of various cancers,[15,16] and gastric cancer.[17] Although several studies have investigated the utility of CEUS for the diagnostic evaluation of lymphomas,[20–25] studies evaluating CEUS for early response evaluation in lymphomas are exceedingly rare. Little has been known that chemotherapy causes changes in the haemodynamics of lymphoma and that the changes themselves are directly linked to therapeutic effects. A study performed by Wei *et al.*[26] revealed that a decrease in peak intensity was associated with treatment response and survival outcomes in 42 patients with aggressive B-cell lymphoma who were evaluated after two cycles of R–CHOP. Another study by Xin *et al.*[27] showed that a decrease in peak intensity and AUC after the first three cycles of chemotherapy well predicted the overall response in 43 lymphoma patients. The present findings further support that such alterations in intratumour blood perfusions could be detectable using CEUS in a much earlier period during treatment and that changes in these parameters could predict the clinical outcomes in patients with lymphoma. In addition, unlike CT/PET, because CEUS is low-cost, portable, and free of radiation exposure, it can be used frequently, which is an advantage for patients with severe lymphoma.

The role of interim imaging analysis during the treatment of lymphomas has been considered, but optimal timing remains unclear. In contrast to most studies on interim PET whereby it was usually performed after 2–4 cycles of chemotherapy, the novelty of the present study is that interim imaging analysis was used during the early treatment period of lymphoma (i.e., 1 week after the treatment initiation). This period is one of the earliest reported in studies of lymphoma prognosis. Another preliminary study of 20 lymphoma patients conducted by Horger et al.[28] focusing on image changes in 1 week used whole-body diffusion-weighted magnetic resonance imaging and the apparent diffusion coefficient (ADC) was calculated at baseline and within a median of 7 days after therapy onset. ADC values in the responder group increased significantly after the initiation of chemotherapy, whereas those in the non-responder group remained unchanged. In addition, there have been several studies on other tumours, which reported that changes in CEUS imaging within 1 week could predict response to chemotherapy. Response to bevacizumab could be predicted via perfusion parameters obtained using CEUS on day 3 in hepatocellular carcinoma[12] or on day 7 in several metastatic cancers.[16] These findings also support the present hypothesis that evaluating metabolic and/or vascular alteration at 1 week after the

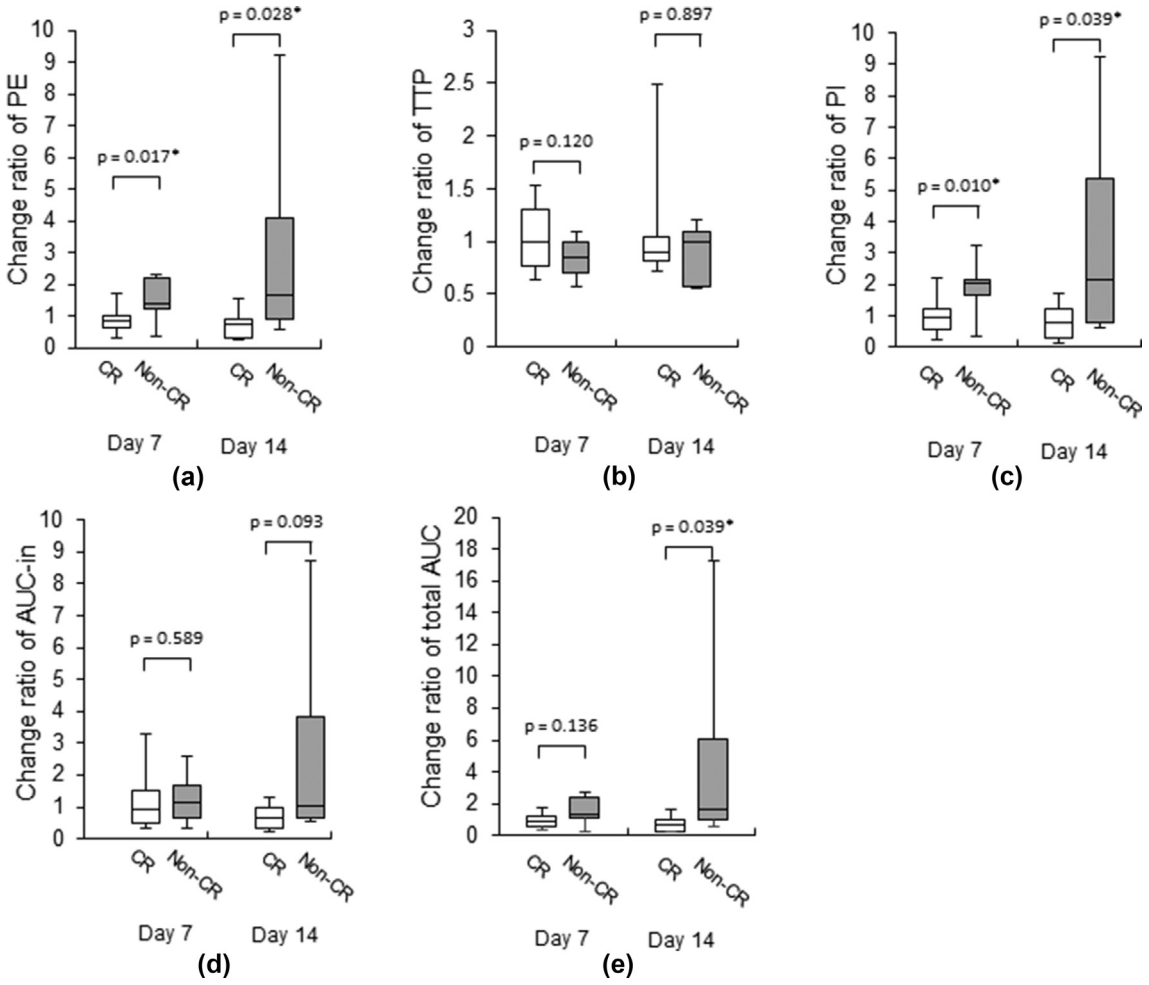


**Figure 3** Change ratios of perfusion parameters compared to the baseline. Change ratio of (a) PE, (b) TTP, (c) PI, (d) AUC-in, and (e) total AUC.

treatment initiation may not be too early for interim analysis for predicting long-term clinical outcomes of malignant tumours. Interestingly, most patients in the non-CR group in the present study showed increased intratumour blood volume and velocity only at 1–2 weeks after the treatment. Furthermore, in the non-CR group, there were some cases in which even if such haemodynamic changes were shown at CEUS, CT/PET was evaluated as a PR.

In conclusion, the present findings suggest that conducting CEUS evaluations during the early treatment period could be useful for predicting long-term clinical outcomes in patients with lymphoma and has the advantage of low

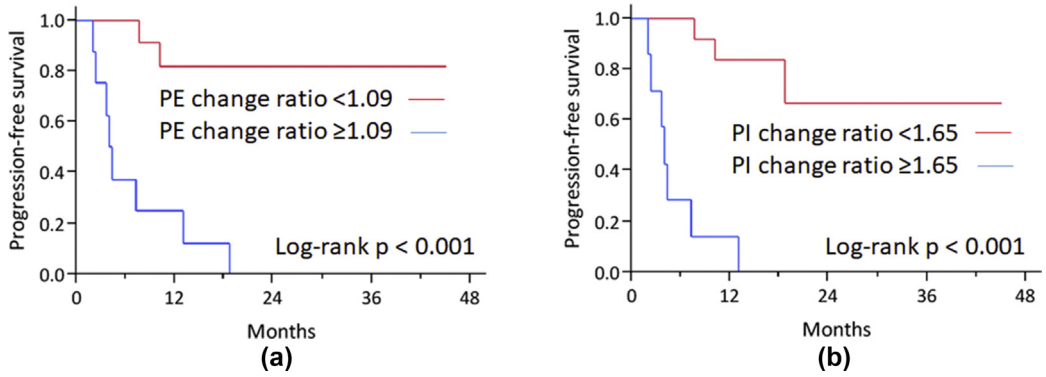


**Figure 4** PFS according to change ratio of PE on day 7 to PE on day 0. (a) The PE change ratio <1.09 group had significantly better PFS than the PE change ratio ≥1.09 group. PFS according to change ratio of PI on day 7 to PI on day 0. (b) The PI change ratio <1.65 group had significantly better PFS than the PI change ratio ≥1.65 group.

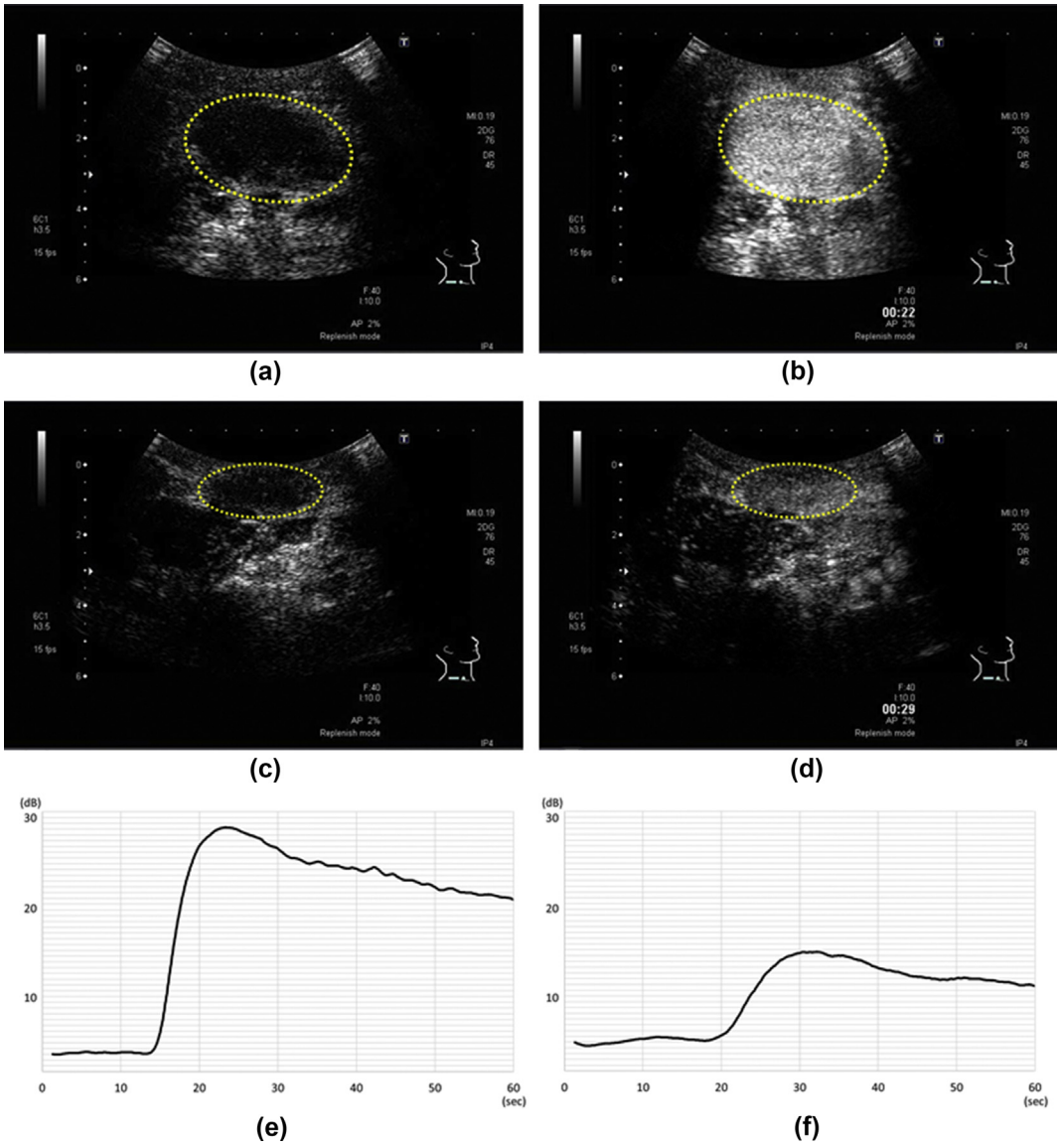


**Figure 5** Clinical example from the CR group. Targeted right neck lymph node in a 63-year-old female patient with anaplastic large cell lymphoma Stage IV treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone. The treatment response was evaluated as PR by computed tomography after 3 weeks. (a) CEUS images of target lesion measuring 43 × 21 mm before treatment, (b) strong vascularisation after bolus injection of perfluorobutane microbubbles before treatment, (c) target lesion measuring 26 × 12 mm on day 7, and (d) strong vascularisation after bolus injection on day 7. The reduction rate was −47.62% and treatment response evaluated as PR using CEUS. (e) TICs acquired via perfusion analysis of this lesion before treatment (parameters: PE; 27.32 dB, TTP; 9.47 seconds, PI; 2.89 dB/s, the AUC-in; 2,610.42, the total AUC; 15,400.57) and (f) day 7 (parameters: PE 10.7126 dB, TTP 12 seconds, PI 0.89 dB/s, AUC-in 1,118.23, the total AUC 4,961.60). This case still remains a CR.

costs and no radiation exposure. Besides, as a result of further studies, if CEUS is judged to be ineffective 1 week after the treatment, switching to another treatment immediately may improve the prognosis of lymphoma.

There are some limitations to the present study. The number of samples in the current study was insufficient to conduct additional analysis, and thus further investigations are required to clarify the relationship between the increase of blood flow and poor response to therapeutic effect. Moreover, the present study included only a small number of patients with heterogeneous characteristics and treatments. Therefore, the changes in CEUS before and after chemotherapy could not be considered separately for HL and NHL. Similarly, the present study did not analyse differences in CEUS imaging between B-cell lymphoma and T-cell lymphoma and between indolent lymphoma and aggressive lymphoma, before and after chemotherapy. Multicentre prospective studies involving a larger number of patients are necessary to confirm and expand the clinical implications of the present findings. Finally, there remains a

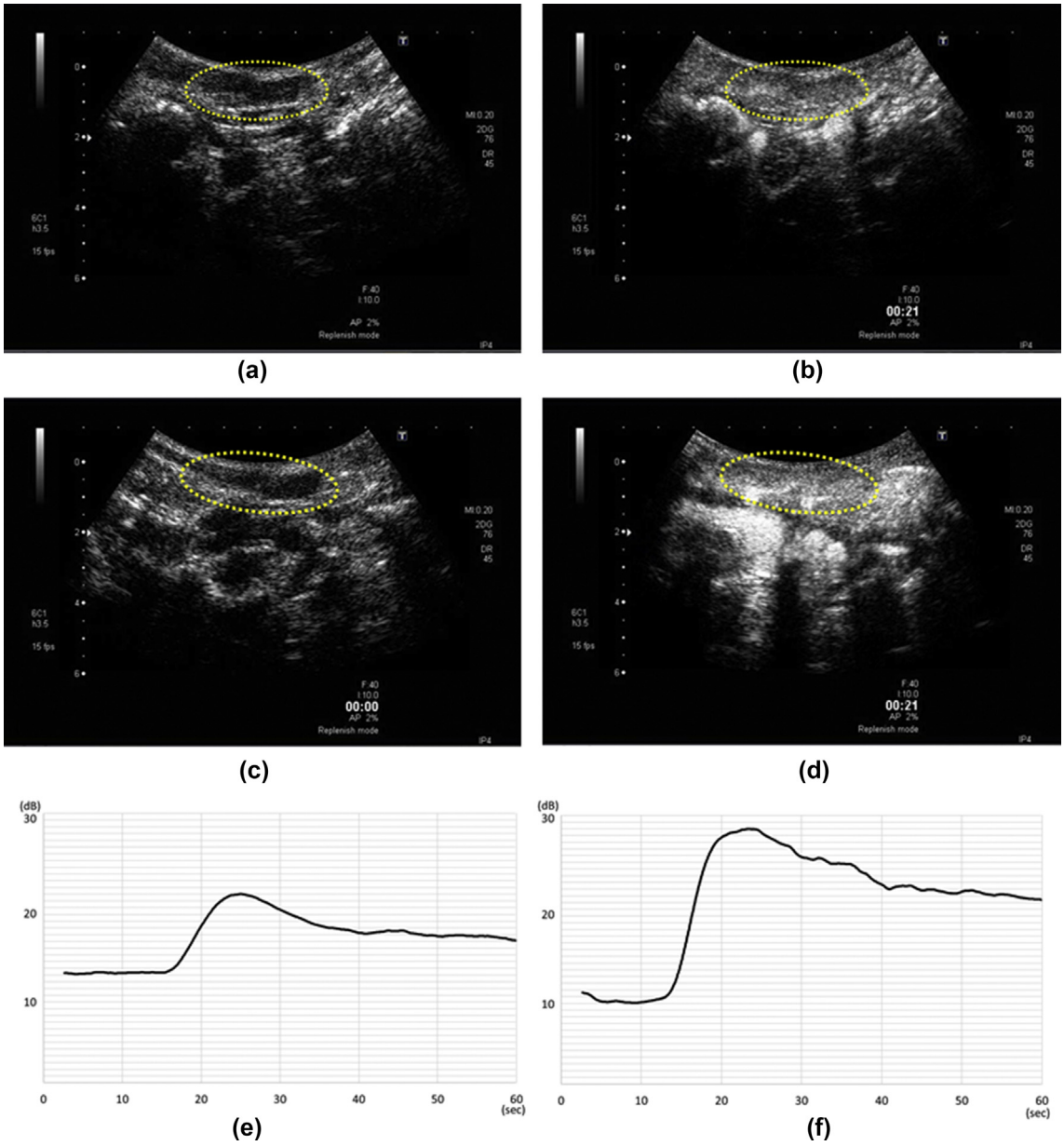


**Figure 6** Clinical example of non-CR group. Targeted right neck lymph node in a 49-year-old male patient with anaplastic large cell lymphoma Stage IV treated with cyclophosphamide, doxorubicin, vincristine, and prednisolone. The treatment response was evaluated as SD by computed tomography after 4 weeks. (a) CEUS images of target lesion measuring 19 × 8 mm before treatment, (b) strong vascularisation after bolus injection of perfluorobutane microbubbles before treatment, (c) target lesion measuring 16 × 6 mm on day 7, and (d) strong vascularisation after bolus injection on day 7. The reduction rate was −12.5% and treatment response evaluated SD by CEUS. (e) TICs acquired via perfusion analysis of this lesion before treatment (parameters: PE 8.21 dB, TTP 8.53 seconds, PI 0.99 dB/s, the AUC-in 588.20, the total AUC 3,437.05) and (f) day 7 (parameters: PE 18.09 dB, TTP 9.27 seconds, PI 1.95 dB/s, AUC-in 1,526.76, the total AUC 9,117.30). This patient died 16 weeks later.

point at the issue of interobserver agreement. Razec *et al.* reported excellent interobserver agreement of whole-body computed tomography for staging and treatment assessment in lymphoma according to the Lugano classification[29]; however, the US imaging technique depends largely on experience. So, it is difficult to numerically prove inter- and intra-observer variability. Therefore, it may be difficult to reproduce these assessments unless the facility has physicians with high experience for US, above all for lymphoma.

## Conflicts of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr. Matsumoto received personal fees from Daiichi-Sankyo and Eisai. Dr. Miura received personal fees from Chugai, Kyowa Kirin, and Nippon Shinyaku. Dr. Ogawa received personal fees from Daiichi-Sankyo, and Canon Medical Systems Corporation. Dr. Nakagawa received personal fee from

Bristol-Myers Squibb. Dr. Takahashi received personal fees from Chugai, Kyowa Kirin, Bristol-Myers Squibb, and Eisai. Prof. Hatta received personal fees from Chugai, Kyowa Kirin, Bristol-Myers Squibb, and Eisai. Prof. Takei received; personal fees from Daiichi-Sankyo, Shionogi, Nippon Kayaku, Chugai, Kyowa Kirin, Nippon Shinyaku, Nichi-iko, Eli Lilly, Bristol-Myers Squibb, and Eisai; and research grants from Daiichi-Sankyo, Shionogi, Nippon Kayaku, Chugai, Kyowa Kirin, Nippon Shinyaku, Nichi-iko, Eli Lilly, and Eisai. Prof. Moriyama received; personal fees from Daiichi-Sankyo, Canon Medical Systems Corporation, Shionogi, Chugai, Kyowa-Kirin, and Eisai; and research grants from Daiichi-Sankyo, Shionogi, Chugai, Bristol-Myers Squibb, and Eisai. All remaining authors have declared no conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crad.2021.02.007.

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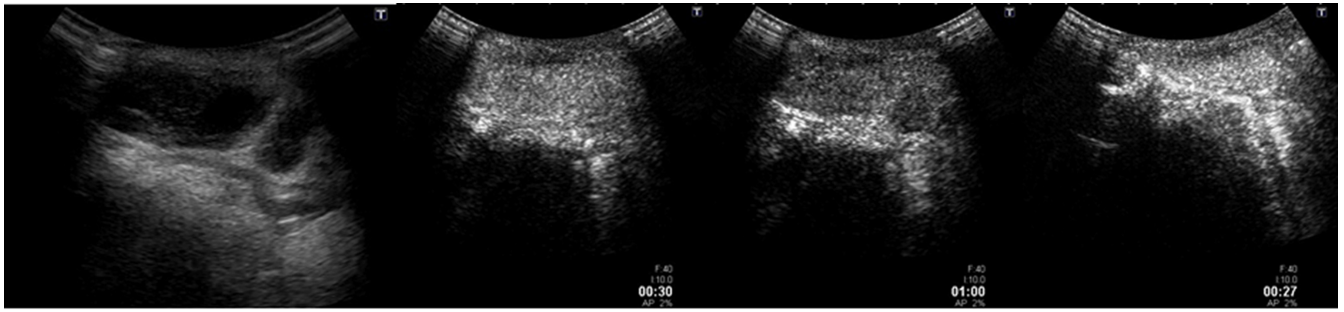
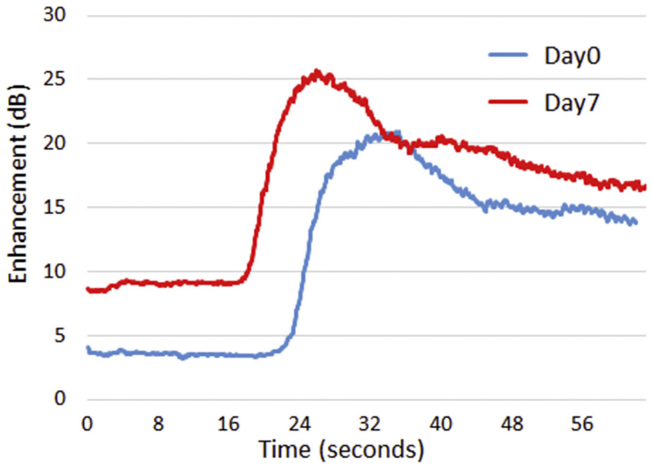
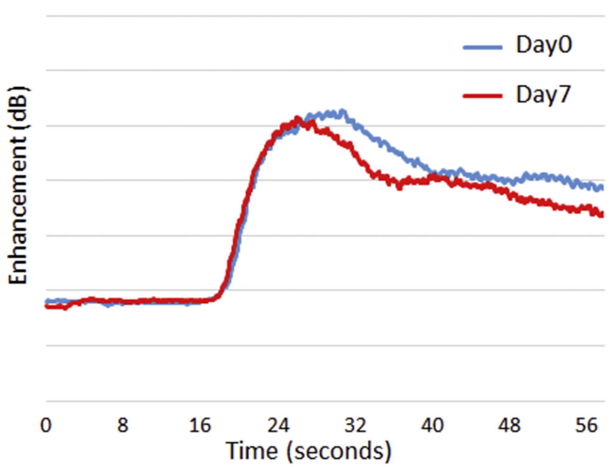
# Supplementaly Figure 1




(a) (b) (c) (d)




(e)




(f)

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Correlation between alterations in blood flow of malignant lymphomas after induction chemotherapies and clinical outcomes: A pilot study utilizing contrast-enhanced ultrasonography for early interim evaluation of lymphoma treatment
（化学療法導入後の悪性リンパ腫の血流変化と臨床転帰の相関：造影超音波検査を用いたリンパ腫治療の早期中間評価のためのパイロット研究）

【目的】　悪性リンパ腫は世界で 7 番目に多い癌であるが、化学療法によく反応する悪性腫瘍でもある。通常、悪性リンパ腫の治療前の病期分類と治療効果判定には CT または PET が用いられる。しかし、放射線学的に得られるサイズの変化は、治療効果判定のタイミングとしては遅い。近年、早期に治療効果判定をするためのモダリティとして中間評価の PET（interim-PET）が導入されている。ただし、CT や PET を頻回に使用する場合、放射線被曝と高コストが懸念される。
一方、造影超音波検査（CEUS）は放射線被爆もなく、低コストであり、肝細胞癌をはじめとする悪性腫瘍の分子標的薬療法の効果判定に有用である。この研究の目的は、悪性リンパ腫治療における化学療法に対する反応の早期中間評価としての CEUS の有用性を明らかにすることである。

【対象と方法】　当院血液膠原病内科に入院し、悪性リンパ腫に対し化学療法を施行した患者を対象とした。当院の臨床研究審査委員会の承認のもと（RK-13028-1）、被験者からの Informed consent を得た。治療前と治療後（7 日目および/または 14 日目）に選んだ標的病変に対し CEUS を行い、従来の評価方法として、治療前と予定の化学療法終了後に CT または PET を行った。CT または PET の効果判定基準に従って、完全寛解（CR）達成群と非 CR 達成群の 2 群に分類した。CEUS の動画から時間強度曲線（TIC）を作成し、評価パラメータとしてピークの造影強度（PE）、ピークまでの時間（TTP）、造影増加速度（PI）、ピークまでのグラフ下の面積（AUC-in）、および 60 秒までのグラフ下の面積（AUC）を抽出し、それらを二群間で比較した。有意差のあったパラメータの変化率に対し、CR を予測しうるカットオフ値を求め、無増悪生存期間の違いの比較を行った。

【結果】　2013 年 2 月から 2017 年 6 月までに当院血液膠原病内科で化学療法を施行した悪性リンパ腫のうち 27 例が本研究の対象となった。 CT または PET による効果判定で 18 例が CR 達成群、9 例が非 CR 達成群であった。観察最終日までの超音波 B-mode でのサイズの減少率の中央値は CR 群 49%、non-CR 群 33%であり、CR 群で大きかったものの、二群間に有意差は認めなかった（p=0.491）。
7 日目までの PE と PI の中央値の変化率に、二群間で有意差を認めた（PE：0.81 vs 1.39、p＝0.017、PI：0.92 vs 2.09、p＝0.010）。CR を予測するためのカットオフ値は

PE 変化率＜1.09（特異度：86%、感度：88%）および PI 変化率＜1.65（特異度：86%、感度：94%）であった。PE 変化率＜1.09 または PI 変化率＜1.65 を達成した患者は、無増悪生存期間が有意に良好であった（p＜0.001）。

【考察】　これまで、悪性リンパ腫治療における早期の治療効果判定の概念は確立されておらず、化学療法によって悪性リンパ腫の血行動態に変化が生じること、その変化が治療効果に直結する可能性があることについては、あまり報告がない。interim PET や MRI の有用性の研究は数多くあるが、治療後 1 週間はその中でも最も早期の効果判定であった。また、造影超音波は、低コストで機動性が高く放射線被爆がないため、重症度の高い症例でも、同一入院中に頻回に評価することが可能であった。
本研究の結果から、CEUS によって抽出されたパラメータが CR 予測に有用であることが示された。血流に関するパラメータは時間関連（blood flow：流入血流速度）と造影強度関連（blood volume：流入総血流量）の二種に分類される。本研究においては、TTP が時間関連、PE、AUC が造影強度関連にあたる。PI、AUC-in は両方の要素を含んでいるが、PI は PE/TTP なので、造影強度をより強く反映しており、AUC-in は PE×TTP/2 に近似されるため、時間も造影強度も反映しにくい。本研究で有意差を認めた PE、PI は造影強度を反映したパラメータである一方、TTP、AUC-in では有意差を認めなかった。つまり、造影強度関連のパラメータが治療効果判定に有用であり、病変内の流入総血流量が治療効果と関連している可能性が高い。
標的病変サイズの減少率は二群間で有意差を認めなかったが、治療によりほとんどの症例で病変は縮小した。病変サイズに変化のなかった症例を non-CR 群に 2 例認めたが、いずれも PE、PI はカットオフ値を超えており、血流の変化が予後予測に有用であったといえる。
本研究は CR を予測する因子を抽出するために CR/non-CR 群間での比較を行った。化学療法の予定サイクル施行後の造影 CT/PET による効果判定結果は、27 例中、CR が 4 例、PR が 14 例、SD が 9 例で、PE、PI の中央値はそれぞれ、CR が 0.89、1.07、PR が 0.94、0.91、SD が 1.39、1.65 で、有意差はないものの PE には予後の悪い判定になるにつれ数値が大きくなる傾向がみられた。
本研究で得られたカットオフ値のみで治療方針を決定していくには、さらなる検討が必要である。PE のカットオフ値を用いて予後良好群と割り振られた 22 症例のうち 17 症例が CR 群、5 例が non-CR 群であり、予後不良群と割り振られた 5 症例のうち 1 例が CR 群、4 例が non-CR 群であり、PFS の分離がいいとはいえない。一方 PI は、カットオフ値を用いて予後良好群と割り振られた 17 症例のうち 16 症例が CR 群、1 例が non-CR 群、予後不良群と割り振られた 10 症例のうち 2 例が CR 群（2 例とも再発、うち 1 例はその後死亡）、8 例が non-CR 群で、PFS の分離は良好であった。ただし、全 27 症例の PE、PI の数値の治療前後の変化を、CR 群と non-CR 群に分けてみると、

CR 群では一部例外はあるものの、PE、PI ともに継時的に低下傾向～横ばいであり、non-CR 群では PE、PI ともに増加傾向であった。現時点でカットオフ値のみでの実臨床への応用は難しいが、interim-PET の結果などと併せて、症例ごとの PE、PI の治療前後の変化を治療方針の変更の一助として活用できる可能性がある。
今回、non-CR 群 9 例の中で、2nd line の治療となった症例は 5 例で、最終転帰は 3 例が死亡、1 例が PR、1 例が PD であった。いずれも 1st line の治療完遂後に 2nd line に移行しており、1st line の途中で治療を切り替えている症例はなかった。本研究の結果を用いて、より早期に治療計画を変更することが可能になれば、今後の悪性リンパ腫の予後の延長を期待できると考える。
本研究で、標的病変の選択には診断時の造影 CT/PET を参照し、その中から超音波で描出可能な病変を選択した。肝臓の一部や体表から遠い深部、骨や頭蓋内は超音波では描出不可能な部位になるからである。本研究の標的病変には節外性病変も含まれるが、超音波検査における悪性リンパ腫の所見には「境界明瞭な低エコーな多血性腫瘤で、微細な血流シグナルを認める」という特徴があり、その特徴は節性病変でも節外性病変でも同様である。節性病変も節外性病変も、腫瘍細胞が密に存在している悪性リンパ腫の病理学的特徴は共通しており、超音波所見はそれを反映しているため、臓器による違いは影響しないと考える。
CEUS は検査者の技術に依存する部分が多いため、安定した技術で検査ができる者を増やしていかなければ、汎用性を高めることは困難である。また、本研究は症例数が少ないために、病型分類や臨床分類による血流の変化の違いを比較できていない。悪性リンパ腫は病型分類によって病理学的に血流量の差がそもそもあるため、血流の減少率・増加率も病型分類によって異なる可能性がある。CEUS による早期治療効果判定のエビデンスを確立していくためには、現状で interim-PET の研究が進んでおり、早期治療効果判定の有用性が高いと考えられる Hodgkin リンパ腫、濾胞性リンパ腫、びまん性大細胞性 B 細胞リンパ腫に症例を絞り、さらなる症例数の蓄積と検討が必要と考える。さらには全身性疾患である悪性リンパ腫において、標的病変 1 か所のみの評価が不十分である可能性は大いにある。本当に局所の血流評価で十分であるかという点についても、検討を重ねる必要がある。

【結論】
本研究では、CEUS で評価した治療開始 1 週間後の TIC のパラメータの変化率が、悪性リンパ腫患者の CR 達成群と非 CR 達成群の間で有意に異なることを示した。それらのパラメータを用いて、CR の有無、PFS を予測可能であった。CEUS を用いて抽出された治療後早期のパラメータの変化は、その後の治療方針の一助にすることで、悪性リンパ腫患者の予後を改善する可能性があると示唆された。